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SHORT REPORT

Monitoring *Aspergillus* species by quantitative PCR during construction of a multi-storey hospital building

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Aspergillus; Hospital enlargement; QPCR; Construction; Monitoring

Summary During the enlargement of an existing hospital, quantitative polymerase chain reaction (PCR) was used to monitor *Aspergillus* spp. populations within the construction site. The rapid availability of results meant that the construction schedule was largely uninterrupted, while assuring that the new construction was free from contamination by the targeted *Aspergillus* spp.

Introduction

Nosocomial fungal infections represent a persistent threat in hospitals especially during construction and renovation.[1,2] One of the major issues in fungal environmental control has been monitoring these fungi in a timely manner. Quantitative polymerase chain reaction (QPCR) allows for the rapid (2-4 h), sensitive (often down to a single spore) detection and quantification of fungi.[3] In this report, we describe the application of this technology to the analysis of *Aspergillus* spp. contamination of a multi-story extension of a community hospital in the upper mid-west of the US.

Material and methods

Early in the construction process, air and dust samples were collected on floors 1, 2 and 3, after the building materials on these floors had been exposed to rain water. An operating room (OR) and a neonatal intensive care unit (NICU) were to be located on the second floor. Samples were also collected in these areas during and after building work.

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Table I *Aspergillus* concentrations in the air on different floors of the new hospital building on 29 January 2002

Aspergillus species	Number of cells/m^3 air			
	Outdoors	Floor 1	Floor 2	Floor 3
Aspergillus flavus	<2	<2	4	<2
Aspergillus fumigatus	<2	<2	<2	<2
Aspergillus niger	<2	<2	980	<2
Aspergillus ochraceus	<2	<2	<2	<2
Aspergillus sydowii	<2	<2	45	<2
Aspergillus ustus	<2	<2	380	<2
Aspergillus versicolor	<2	<2	<2	<2
Eurotium (Asp.) spp.	<2	27	2900	<2

Air samples were collected on 0.45 μm pore size polycarbonate filters (Osmotics Inc., Minnetonka, MN, USA) in three piece cassettes, at 3 l/min for 3 h. Surface dust samples were collected using a 37 mm cassette (Zefon International, Inc., St Petersburg, FL, USA) with a MCE filter and a 1 in. length of tubing attached to the inlet. The cassette was attached to a high volume pump (SKC, Eighty Four, PA, USA) operating at a flow rate of 15 l/min. The cassette was drawn over a 4 ft^2 area in vertical, horizontal and opposing diagonal directions, capped and shipped to a commercial laboratory for QPCR analysis, as previously described.[3]

Results

Table I lists the results of the initial air sampling of the floors of the extension after the visibly contaminated building materials were removed. A more extensive analysis of the new OR and NICU was undertaken in April of 2002 as construction continued on the upper floors.

The QPCR analysis of the samples of floor and surface dust from the new OR is shown in Table II. High concentrations of several *Aspergillus* species, including *Aspergillus fumigatus* and *Aspergillus niger*, were found in both the floor and surface dust samples. Several terminal cleanings were performed. These consisted of cleaning the floors and walls using mechanical friction with hospital-grade quaternary ammonia disinfectant. Eventually, the aspergillus contamination was eliminated (Table II).

The new NICU floor was also contaminated with a number of *Aspergillus* species, including *Aspergillus flavus* (Table III). Sampling of the corridor carpet, suggested that this carpet might be the source of the contamination in the NICU (Table III). Extensive high-efficiency particulate air (HEPA) filter vacuuming of the carpet was undertaken. Subsequent sample results revealed a reduction in the aspergillus burden. HEPA filter vacuuming was performed once a shift (three times daily) for a week and then once daily for the next seven months. *Aspergillus* spp. levels in carpet were greatly diminished by this regimen, approaching non-detectable levels at the end of this process (Table III). This resulted in the removal of the targeted *Aspergillus* contamination of the NICU floor.

Discussion

Nosocomial aspergillus infections cause many deaths each year and remain a major health problem.[4] Traditionally, fungal populations in hospitals are estimated by culturing on various media, but the results can take days to weeks to obtain. In

Table II Average *Aspergillus* concentrations in surface dust in newly constructed operating room

Aspergillus species	Average number of cells						
	Floor				Surface		
	09/04/2002[a] $N=2$	19/04/2002[a] $N=4$	29/04/2002[b] $N=4$	21/11/2002[b] $N=2$	19/04/2002[a] $N=2$	29/04/2002[b] $N=2$	21/11/2002[b] $N=2$
Aspergillus flavus	<1	1	5	<1	395	10	<1
Aspergillus fumigatus	345	36	44	<1	915	<1	<1
Aspergillus niger	140	15	126	<1	2050	100	<1
Aspergillus ochraceus	<1	<1	<1	<1	<1	<1	<1
Aspergillus sydowii	25	108	<1	<1	2050	80	<1
Aspergillus ustus	20	21	<1	<1	2956	190	<1
Aspergillus versicolor	125	191	125	<1	1050	28	<1
Eurotium (Asp.) spp.	700	1280	603	<1	7504	227	<1

[a] Reported as average number of cells per mg dust.
[b] Reported as average number of cells per sample since insufficient dust was collected over the sample area. This reflected reduced dust loading after repeated HEPA vacuuming.

Table III Average *Aspergillus* concentrations in carpet dust in neonatal intensive care unit

Aspergillus species	Average number of cells				
	Floor			Corridor carpet	
	09/04/2002[a] $N=2$	29/04/2002[b] $N=3$	21/11/2002[b] $N=2$	19/04/2002[a] $N=1$	21/11/2002[b] $N=1$
Aspergillus flavus	119	<1	<1	11	3
Aspergillus fumigatus	<1	23	<1	660	<1
Aspergillus niger	50	74	<1	380	<1
Aspergillus ochraceus	<1	<1	<1	<1	<1
Aspergillus sydowii	30	<1	<1	390	<1
Aspergillus ustus	398	117	<1	220	3
Aspergillus versicolor	145	158	<1	1400	<1
Eurotium (Asp.) spp.	1495	1517	3	19000	55

[a] Reported as average number of cells per mg dust.
[b] Reported as average number of cells per sample since insufficient dust was collected over the sample area. This reflected reduced dust loading after repeated HEPA vacuuming.

addition, analysis of samples by QPCR and by traditional culturing has shown that fungal populations are underestimated by the traditional culturing techniques.[5] Another advantage of QPCR is that air sampling is not limited to minutes, as is the case now with culturing. Thio *et al.*[6] demonstrated that large volume air samples are superior to small volume samples in assessing aspergillus in the healthcare environment, but the most important advantage of QPCR is that results can be obtained in a matter of hours.

The rapid availability of the data about aspergillus contamination allowed the consulting engineers to pin-point the contamination on the second floor. Even though the obviously contaminated materials were removed, the QPCR results demonstrated that contamination remained. The QPCR data led to the discovery of other sources and their removal before further construction continued. As a result construction was completed after only a minimal delay and the problem was corrected before occupancy.

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